CANINE PIROPLASMOSIS. VI.

STUDIES ON THE MORPHOLOGY AND LIFE-HISTORY OF THE PARASITE.

(Plates I—III and Diagrams 24—37.)

(*Continued from* Vol. VI., p. 651.)

By GEORGE H. F. NUTTALL, M.D., Ph.D., Sc.D., F.R.S.,
Fellow of Christ's College, Quick Professor of Biology in the University of Cambridge,

and G. S. GRAHAM-SMITH, M.A., M.D.,
University Lecturer in Hygiene, Cambridge.

(*From the Pathological and Biological Laboratories, Cambridge.*)

Introduction.

In the present paper we describe the results of further investigations on the life-history of *Piroplasma canis* in the blood of the dog. In our last paper (x. 1906)[1] we described and figured the movements of the parasite and the mode of multiplication in the dog's blood, and since that time we have confirmed most of our previous observations, and added further facts. The technique of these examinations was fully explained in that paper (p. 604), and it is only necessary to state here that all our later observations on living blood have been made at a temperature of 35°—40° C. The drops of blood were mounted on clean glass slides and cover-glasses kept at this temperature, and were placed as rapidly as possible on the stage of a microscope kept at a similar temperature in a Nuttall's thermostat and examined under a $\frac{1}{12}$th oil immersion lens. In fact we have endeavoured to make our

[1] Nuttall, G. H. F., and Graham-Smith, G. S. (x. 1906), Canine Piroplasmosis. V. Further studies on the structure and biology of the parasite. (Plates XI—XIII, Diagrams 1—23), *Journ. of Hygiene*, vol. VI. pp. 586—651. The bibliography is given in this paper.

observations in such a manner that the blood should be altered as little as possible.

Our examinations of the living blood have been made at all stages of the disease, at all times of the day between 9 a.m. and midnight, and have occupied more that 550 hours. Whenever necessary individual parasites have been continuously kept under observation for long periods, even up to 3 hours or more, especially in the case of forms on which our observations had previously been scanty. We, therefore, feel that we are justified in considering that if any other methods of multiplication occur than those which we are about to describe, they must be extremely rare.

Whenever fresh preparations were being examined thin smears were also made on clean glass slides and fixed in various ways. Aided by the thorough knowledge which we had gained of the various changes in shape which the living parasites undergo during the process of multiplication we have finally been able, by the examination of stained preparations, to demonstrate the accompanying nuclear changes.

In the following pages we describe (*A*) the appearance of parasites in unstained preparations; (*B*) the mode of multiplication of the parasite and the fate of various forms as observed in the living blood; (*C*) the accompanying nuclear changes as ascertained by the study of stained preparations; (*D*) the complete cycle of development within the blood.

In an appendix we give certain observations on the effect of heat on blood corpuscles.

(A) The appearance of parasites in fresh unstained preparations.

In our last paper (1906, pp. 605—609) we described the various appearances seen in fresh preparations of normal dog's blood with special reference to those which are likely to lead to errors in making observations on infected blood. In another place (pp. 635—639) we gave at considerable length our reasons for considering that during certain stages the parasites entered the corpuscles and divided within them. Our further observations have not led us to alter our views on this point. The means of differentiating between intra-corpuscular and epi-corpuscular parasites swimming over the surface of normal corpuscles were given on p. 613. In order to present these differences more clearly we now reproduce three sketches illustrating the appearances

of an intra-corpuscular parasite, when in proper focus, and when not completely in focus, and of a parasite lying on the surface of a normal corpuscle.

Plate I, Fig. 2*b* represents a parasite lying within a red blood corpuscle when properly in focus. It is seen that the edge of the corpuscle is darker than the central portion, and that it is surrounded by a light zone. The parasite appears as a lighter clearly defined body with a dark contour.

Plate I, Fig. 2*c* represents the same corpuscle and parasite when not yet brought into proper focus. In this case the dark and light areas previously noticed are reversed. The corpuscle is surrounded by a dark zone. The corpuscle itself is much lighter in colour and possesses an almost transparent margin. The body of the parasite, especially the marginal third, is darker than the corpuscle and it is surrounded by a light zone.

Plate I, Fig. 2*a* represents a free pyriform parasite lying on a normal corpuscle. In this case as in Fig. 2*b* the corpuscle is a dark body with a darker margin surrounded by a light zone. The parasite also appears as a dark body with a darker margin surrounded by a broad light zone. The latter varies in breadth in different cases, and appears to be due to the attenuation of the substance of the corpuscle owing to the pressure of the parasite upon it.

Plate II, Fig. 50 represents the appearance of a parasite lying on a corpuscle as seen in a stained preparation.

Points already sufficiently dealt with in our last paper.

In our last paper (1906) we fully described with the help of illustrations many of the phenomena which can be observed in the blood of infected dogs. With few exceptions all these changes have been repeatedly observed in our later experiments, and in many instances we find that we have nothing to add to our previous description. The latter include: (*a*) observations on single intra-corpuscular parasites which did not at any time show very marked pseudopodia (pp. 613—616), (*b*) observations on single parasites showing well marked pseudopodia which finally come to rest in a rounded condition (pp. 616—617), (*c*) observations relating to the movements within the corpuscles of various forms of parasites (pp. 618—620), (*h*) observations on the behaviour of certain examples of free pyriform parasites (pp. 625—631), (*k*) observations on the action of leucocytes in the blood of infected

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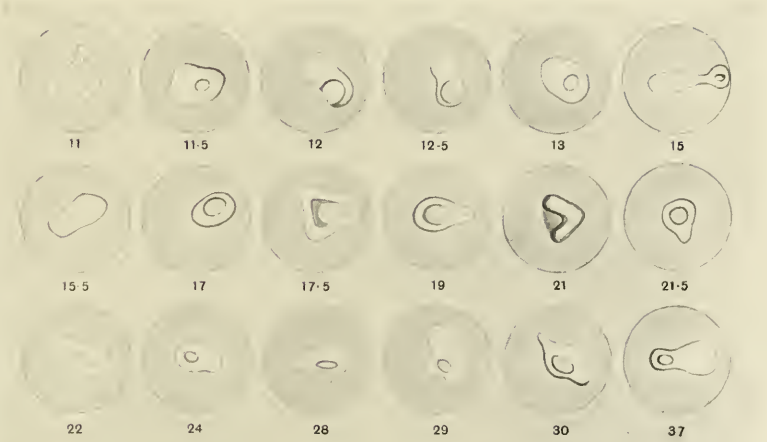
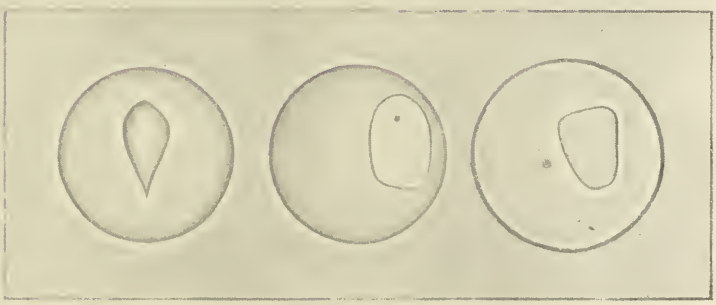
Fig. 1 (11—37).





a *b* *c*

Fig. 2 (*a*—*c*).

dogs (p. 632), (*l*) observations made at night on the living blood (p. 633), (*m*) observations on living blood some hours after the preparation of the specimens (p. 633), (*n*) observations on the living blood within the last few hours of life (p. 634).

In fact, in our later observations we merely supplement those we had already made, except in regard to certain forms illustrated in Diagr. 21, Figs. 3, 4 and 5 of our last paper (p. 627). In this case we were mistaken in our interpretation of the bodies which we saw. These are not parasites, but degeneration products of the red blood corpuscles. Further observations on this point are given in the appendix to the present paper.

(B) The mode of multiplication of Piroplasma canis and the fate of various forms as observed in the living blood.

(1) *The entry of parasites into red blood corpuscles.* Very numerous observations have now been made on the entry of the parasites into red blood corpuscles and we are able to confirm our previous statement (p. 631) that only the pyriform or long parasites enter the corpuscles, never the round forms. The events leading up to the invasion of a normal red blood corpuscle invariably occur in the following order:

Fully mature pyriform parasites, two, four or more, escape from an infected corpuscle and moving with considerable rapidity, sometimes after short intervals of quiescence, approach other corpuscles. When one of these parasites is about to enter a normal corpuscle it generally approaches it with its blunt extremity foremost and rapidly indents its surface. Then violent movement of the thin end of the parasite occurs, and the side of the corpuscle becomes greatly distorted, and it may be caused to oscillate or even be moved from its original position. Gradually the parasite sinks more deeply into the corpuscle and finally disappears within it, when the movements of the corpuscle cease and it resumes its rounded shape. At this time it is generally difficult to define the parasite within the corpuscle, which becomes distinctly darker in colour. Gradually, however, the parasite becomes more visible, and its shape changes from pyriform to oval.

Sometimes, (see Diagr. 33[1], Fig. A, p. 248), these events take place

[1] The Diagrams in our previous paper were numbered 1—23, and, to avoid confusion, we have numbered the Diagrams accompanying this paper 24—37.

with considerable rapidity and with comparatively little distortion of the invaded corpuscle.

Although we have continually borne in mind the possibility of a parasite entering an already infected corpuscle or of two parasites simultaneously entering a normal corpuscle, and have made especially careful and prolonged observations, whenever such events seemed probable, we have never seen either one or the other.

The entry of two parasites into a corpuscle either simultaneously or at different times, must therefore be extremely rare, under the conditions of observations on the slide where the corpuscles are spread out in a thin layer. We cannot, however, on this account be certain that these events do not occur in the body especially in the last stages of the disease, when a considerable proportion of the corpuscles are found to be infected.

(2) *The behaviour of parasites after their entry into normal red blood corpuscles.* Shortly after a pyriform parasite enters a normal corpuscle it changes its shape and becomes rounded and remains quiescent for a variable period (Diagr. 33, Figs. A and B). Most commonly it then grows in size, becomes amoeboid and finally divides into two pyriform parasites (Diagr. 24). More rarely, however, the parasite, while still small, apparently divides into two small rounded parasites, each of which subsequently behaves like a single parasite. The process of multiplication by which a single small rounded parasite becomes converted into two pyriform parasites will therefore be first described.

The formation of two pyriform parasites from a single small round parasite. The small round parasite after a period of quiescence gradually enlarges and shows slight changes in shape. After further growth it becomes actively amoeboid throwing out one or more blunt pseudopodia or delicate processes which are constantly changing their position and shape. Frequently a pseudopodium is retracted and another thrown out (Diagr. 24, Figs. 16—26). The movements of the parasites during this phase have been fully illustrated in our previous paper (1906, pp. 598, 613—619, living examples, Diagr. 14 and 15, and fixed preparations, Diagr. 9 and Plate XI, Figs. 3—9), and need not be further described. After a variable period of time the activity becomes greatly diminished and the parasite assumes a more or less rounded shape without any marked pseudopodia (Diagr. 24. Fig. 30; Diagr. 25. Fig. 21). After a longer or shorter time two minute rounded processes are protruded from two closely situated points on the circumference of the parasite (Diagr. 24. Fig. 30; Diagr. 25. Fig. 23).

Simultaneously the two processes gradually enlarge, particularly at their distal extremities, and become more or less pear-shaped, being attached to the main body of the parasite by relatively narrow necks (Diagr. 24. 32; 25. 30). As the protoplasm of the parasite flows into these processes a time is reached when each of the two processes and the remains of the body of the parasite are of approximately equal size, and the whole parasite has a trefoil appearance (Diagr. 24. 33; 25. 35).

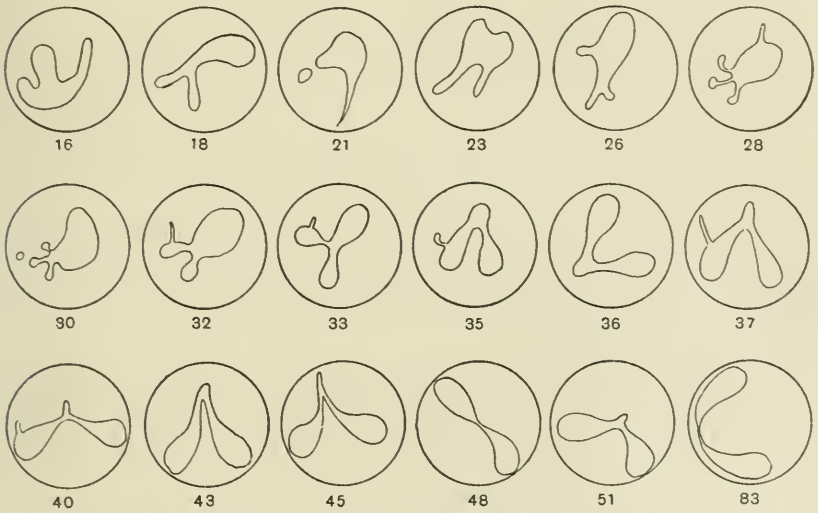


Diagram 24 representing the changes observed during the development of a single amoeboid parasite into two pyriform parasites[1].

After this the simultaneous enlargement of the processes at the expense of the body of the parasite still continues and the latter becomes smaller and smaller, but in the majority of cases still retains its rounded form until it appears as a minute rounded mass to which the processes now converted into almost mature pyriform parasites are attached (Diagr. 24. 35—36; 25. 38). Ultimately this mass completely disappears and the two processes alone remain as two mature pyriform parasites, occasionally joined by a thin strand (Diagr. 24. 37—83; 25. 45).

In the accompanying Diagrams (24 to 26), drawn from typical examples of living parasites, the gradual development of two pyriform

[1] The numbers under the figures indicate the number of minutes which had elapsed after the preparation of the specimen.

parasites from single amoeboid forms can be more easily followed than described.

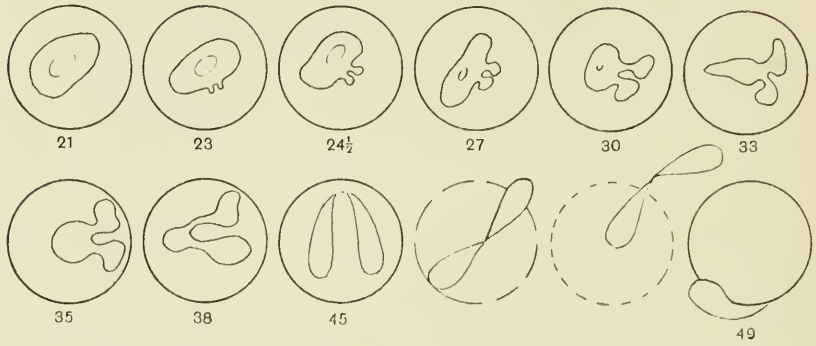


Diagram 25 representing the changes observed during the development of a single rounded parasite into two pyriform parasites.

The complete cycle has not been observed in a single specimen, but the formation of amoeboid parasites from small forms, and the formation of two pyriform parasites from amoeboid parasites have been followed on many occasions.

Although the method just described in which the main mass of the parasite gradually and symmetrically flows into the newly formed processes is by far the most common, and has been observed on very many occasions, variations are sometimes noticed, some of which are illustrated in Diagr. 26, in which many of the intermediate stages are omitted.

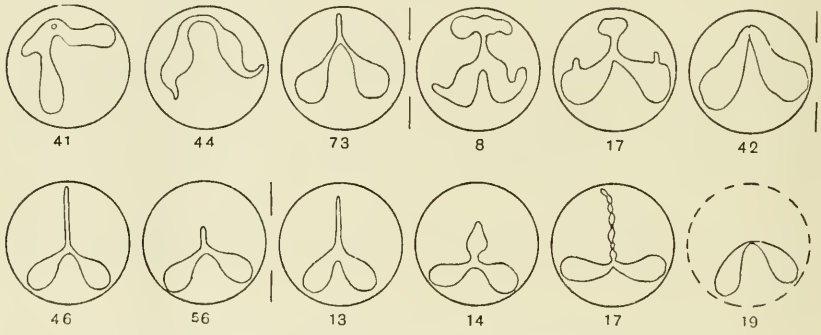


Diagram 26 representing the final stages of development of several parasites into double pyriform bodies.

The first three figures (41, 44, 73) represent the gradual transformation of a single parasite with two irregular processes up to the

stage shown in Diagr. 24. 40. In this case the main mass of the parasite was early absorbed into the process. The parasite was kept under observation for 63 minutes from an early amoeboid stage till the development of two mature pyriforms. The stages of special interest only were picked out for illustration. The next three figures (8, 17, 42) representing the transformation of a parasite from a stage corresponding to Diagr. 25. 33 into two somewhat irregular pyriform parasites, were selected from a series of sketches showing the complete development of an amoeboid parasite during 37 minutes. In the stage shown in Fig. 8, that portion which formed the main mass of the original parasite is seen to be ovoid and attached by a narrow stem to two irregular processes. After a period of 9 minutes (17) the main mass became round and the processes almost pyriform except for the fact that each showed a small projection. After a further period of 25 minutes the original parasite had completely divided into two slightly irregular pyriform parasites. In Figs. 46 and 56, the last phases of the division of a parasite are shown. In this case the main mass, instead of remaining rounded, assumed a rod-like shape, before finally disappearing. In the last four figures a somewhat similar condition is represented. The rod-like projection (13) alternately elongated and contracted before being finally absorbed. At one stage it was long and rod-like (13), at another rounded (14), and later it again became elongated and had a beaded appearance (17). Before its final disappearance it contracted and elongated several times. The last figure (19) represents the two conjoined parasites which were produced, and which ultimately escaped from the corpuscle. The broken contour line of the corpuscle is intended to indicate that the corpuscle faded before the parasites left it.

On very rare occasions we had observed the division of a single amoeboid parasite into four pyriform parasites.

Diagram 27 (3) shows a parasite protruding two pairs of processes. Each pair exactly resembles the pair which in the method just described gives rise to two pyriform parasites. The development was followed for 5 minutes during which the processes rapidly enlarged, and the parasite almost reached the stage represented in the next figure. At this moment the corpuscle unfortunately ruptured, and development came to an end.

Diagram 27 (2, 18, 42) shows, however, the later stages of such a method of division in which four pear-shaped processes joined to a single stem eventually separated into four pyriform parasites.

Occasionally a single small round parasite after passing through an amoeboid stage assumes the typical pyriform shape of the mature form and escapes from the corpuscle.

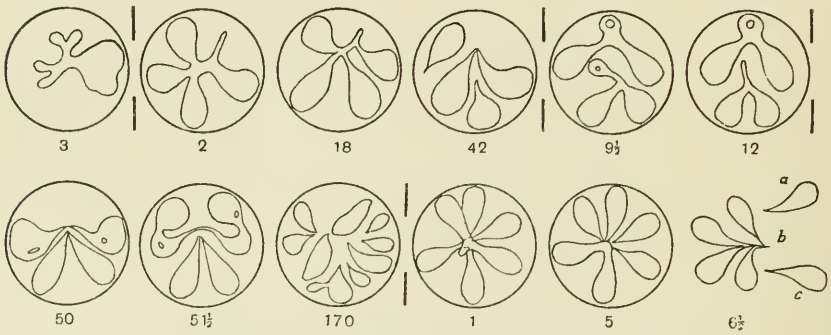


Diagram 27.

(3) *The simple division of small rounded parasites.* Red blood corpuscles containing two small rounded parasites are frequently seen both in fresh and stained preparations, but their origin cannot be easily determined. In some cases they may possibly be derived from the invasion of the corpuscle by two pyriform parasites, though we have never observed such an occurrence, but in most cases they seem to be derived from the simple division of a small rounded parasite.

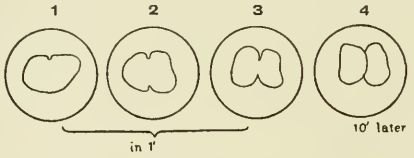


Diagram 28 representing the division of a small rounded parasite by simple division.

Although we have very carefully studied the behaviour of the small round parasites we have very seldom observed satisfactory examples of simple division. Indications of approaching division such as the formation of deep indentations on opposite sides of an elongated parasite are common (Diagr. 28. 1, 2, 3) and frequently end in the apparent division of the parasite into two smaller rounded parasites (4). Some of these appear to be cases of true division. In other cases, however, although no connection can be seen between the apparently divided portions of the parasite, on further observation the two parts again become united, showing that some delicate and

invisible bond had existed between them (Diagr. 29. 14, 15, 15½). Observations on amoeboid parasites show that outlying portions are frequently attached to the main mass by such attenuated strands that they are for a time either almost or completely invisible in living preparations (Diagr. 24. 21).

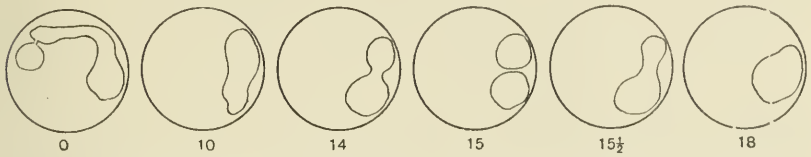


Diagram 29 representing the apparent division (Fig. 15) of a single amoeboid parasite.

Owing to the frequency of these deceptive appearances it is extremely difficult to decide whether true division has occurred or not, unless the corpuscle is kept under observation for a considerable time and the newly divided parasites definitely indicate their lack of connection by their movements.

In spite of these difficulties we are convinced that such division does occur, and this view appears to be confirmed by the examination of stained preparations.

(4) *The behaviour of double amoeboid parasites within single red blood corpuscles, and the formation of four pyriform parasites from them.* The behaviour of two amoeboid parasites within a single corpuscle has been frequently followed out. On many occasions the corpuscle ruptured and the parasites were liberated without any change in form.

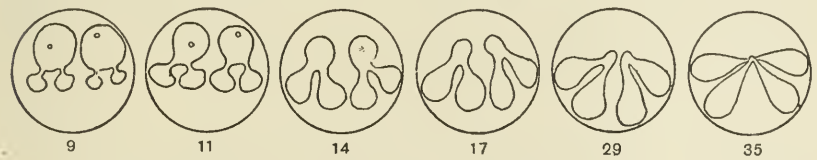


Diagram 30 representing the stages observed during the simultaneous development of two amoeboid parasites into four pyriform bodies.

On several occasions we were, however, able to observe the formation of two pyriform parasites from each of the amoeboid forms. The method is invariably the same as that which is seen in the formation of two pyriform parasites from a single amoeboid form, namely, by the protrusion of a pair of processes from each amoeboid parasite after it had become more or less inactive.

At times the changes occur simultaneously as indicated in Diagr.

30 and 31 whilst at other times one parasite is slightly in advance of the other (Diagr. 27. 9½, 12).

At other times one amoeboid parasite completes its division before the other has ceased to be actively amoeboid (Diagr. 33. 4). Under these conditions we see within an infected corpuscle two mature pyriform parasites and one large round or amoeboid form.

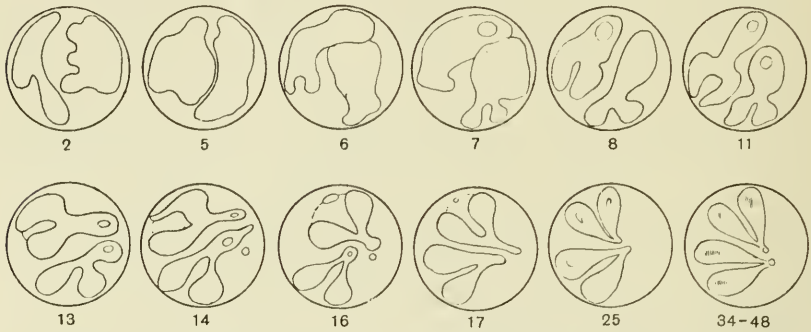


Diagram 31 representing the stages observed during the almost simultaneous development of two amoeboid parasites into four pyriform bodies.

Very rarely the single amoeboid parasite has been seen to assume a pyriform shape, thus giving rise to three pyriform parasites in one corpuscle.

(5) *The formation of several pyriform parasites in a single red blood corpuscle.* Owing to the superposition of parts of one parasite upon another, and the difficulty of following rapid movements in a corpuscle containing several parasites, it is an extremely difficult matter to clearly distinguish the formation of several pyriform parasites. Occasionally, however, the process can be followed.

Diagram 27 (1, 5, 6½) represents the later stages in the formation of six pyriform parasites in a single corpuscle. When first noticed, immediately after the preparation had been mounted, three almost completely differentiated pairs of pyriform parasites, all in the stage illustrated in Diagr. 24. 40 were seen, with their connecting parts overlapping. Within 5 minutes one pair had become differentiated into two completely separate pyriform parasites. Shortly afterwards the corpuscle ruptured and two free parasites and four joined to a single stem were liberated. The latter had therefore probably been formed from one amoeboid parasite as illustrated in Diagr. 27 (2, 18, 42).

The subsequent behaviour of these free parasites was watched for some time. One of the single parasites swam away and after a few

minutes could not be followed, the other became round and still after 27 minutes. The four conjoined parasites appeared to attempt to swim in different directions causing the whole mass to vary slightly in position. After 21 minutes they were engulphed by a leucocyte.

Other instances of a similar kind have also been noticed showing that the same method of division is followed in the formation of multiple pyriform parasites within a single corpuscle as in the formation of a pair.

In another case two pyriform and two irregular amoeboid parasites were noticed within a corpuscle (Diagr. 27. 50—51½). The specimen was watched for some time but no further development was noticed. The preparation was however left under the microscope and examined 2 hours later. It was then seen that the irregular parasites had undergone division, and that the corpuscle then contained eight more or less regular pyriform and one irregular pyriform parasite with a process projecting from its side. No doubt could be entertained as to the identity of the corpuscle since no other infected corpuscles were near it.

(6) *Irregular pyriform parasites.* Our observations on living and stained preparations have shown that a small proportion of the parasites resulting from the division of single or double amoeboid forms are not typical pyriform parasites, but pyriforms showing certain irregularities in shape.

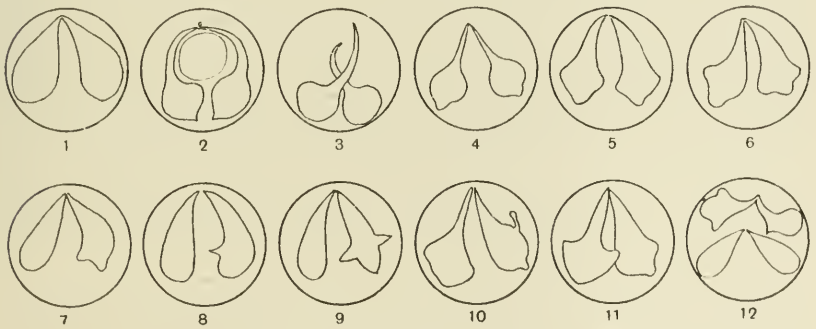


Diagram 32.

Diagram 32 illustrates a number of these forms. Fig. 1 shows a very large but otherwise regular type, Figs. 2 and 3 types with rounded bodies and long curved tails, Fig. 4 a similar type with a straight tail, Figs. 5—12 illustrate pyriform types with blunt angles or

pointed processes projecting from the sides of the otherwise pyriform parasites.

All these types are peculiar in that they seldom show any movement within the containing corpuscles[1], and with the exception of the types with the curved tails (2 and 3), even when freed by the rupture of the corpuscle remain stationary. The latter move about in a sluggish manner by means of slow movements of their tails, but never seem to attack corpuscles and soon become motionless. When free in the plasma all these forms remain in the same condition and do not disintegrate for a considerable time.

We have never seen any of these forms enter fresh corpuscles, although we have made repeated observations upon them spending many hours in the study of these forms alone.

Occasionally the pear-shaped processes, which ultimately develop into typical pyriform parasites, exhibit for a time spike-shaped lateral processes as seen in Diagr. 24, which are finally withdrawn. Some of the parasites shown in Diagr. 32 may therefore represent organisms whose development has been arrested.

(7) *The escape of the parasites from infected red blood corpuscles.* In our later experiments we have confirmed and extended our previous observations (p. 622) on the escape of parasites from infected corpuscles. Parasites have now been seen in the act of leaving the corpuscles on many occasions, and it has been noted that three different methods may be adopted. (*a*) Most commonly the parasite or parasites leave the corpuscle, and the latter immediately becomes pale and finally disappears. (*b*) Less commonly the corpuscle first becomes pale and then the parasite escapes. (*c*) On very rare occasions the parasite appears to leave the corpuscle without apparently injuring it.

(*a*) The mode of escape about to be described in detail is undoubtedly the most common one under experimental conditions, and satisfactorily accounts for the watery condition of the blood and the haemoglobinuria during the last hours of life.

An intra-corpuscular parasite when properly focussed appears as a well defined light body surrounded by a narrow darker zone within the dark corpuscle (see p. 233). No intervening light halo is seen. At times, prior to the escape of the parasite, without any apparent disturbance of the surface of the corpuscle, the parasite seems to disappear as into a fog. Though its general form can still be defined,

[1] We have on many occasions kept such intra-corpuscular forms under observation for hours.

its outline is no longer sharp, and the colour of the organism approaches more nearly to that of the corpuscle. This appearance may be due to the corpuscle assuming a more spherical form owing to the absorption of fluid. Gradually, or at other times rapidly, the parasites become more distinct, show active movements and simultaneously pass out of the corpuscles, often without apparently encountering any great resistance, and swim away. The corpuscle then rapidly loses its colour and almost disappears, although its margin can be still defined by careful focussing. Occasionally, however, no apparent trace of it remains.

Slight differences are noticed in various cases, for example, the foggy stage may not be observed, or the organisms may distort the corpuscle to some extent before their escape as if the envelope offered considerable resistance. At other times the process is so rapid that the various changes can scarcely be followed, and gives the appearance of the parasites being hurled out by the explosion of the corpuscle.

(*b*) On many occasions the corpuscles were noticed to become pale, or almost invisible before the escape of the parasites. Under these conditions the parasites frequently perform remarkable gyratory movements before leaving the remains of the corpuscle, two instances of which we previously described (x. 1906, p. 623).

While in some cases the parasite seems to encounter very little resistance in passing through the remains of the corpuscular envelope, in other cases it meets with sufficient resistance to alter its shape. A parasite has, for example, been noticed to behave like a blood corpuscle passing through a narrow capillary. A portion of the blunt end apparently passes through an aperture in the corpuscular envelope and the organism gradually forces its way through, without increasing the size of the opening. Consequently that portion of the parasite which is encircled by the walls of the opening is constricted and the whole organism has an hour-glass shape (see Diagr. 35. 15).

In connection with these two modes of escape two other appearances of some interest have been noted.

As the corpuscle is fading the surrounding plasma often becomes coloured for an instant on one side of the corpuscle, as if the fluid contents tinged with haemoglobin were being expelled from an opening at the side of the corpuscular envelope. In certain stained preparations the same condition was noticed, confirming the observations made on fresh blood (see Plate III).

It was also frequently noticed that after the fading of the corpuscle

certain very small colourless bodies were seen besides the parasites. These sometimes remained within the contour of the faded corpuscles, were sometimes thrown out into the surrounding plasma, or more rarely appeared to be connected to the parasites.

Up to the present we have been unable to decide on the true nature of these bodies. We were at first inclined to believe that they were residual bodies thrown off from the parasites in the process of division. The fact, however, that they have not been recognised in stained preparations is against this view. On the other hand, it is possible that they represent the remains of the stroma of the corpuscle broken up by the movements of the parasites. These minute bodies, whatever their nature, were found to be present in a considerable number of the cases observed, but in other cases, though looked for with the greatest care, not a trace of them could be seen.

(*c*) In our last paper we described two instances in which parasites apparently escaped from affected corpuscles without causing their destruction (p. 623), but at the same time we pointed out that both these instances might have been accounted for by errors of observation (p. 625). Only two other examples of this mode of escape have been noticed. In one case two pyriform parasites, evidently produced in the usual manner, escaped from opposite sides of the corpuscle which was distorted but soon regained its rounded form, and did not fade. Its border however showed slight undulations. In the other case four pyriform parasites escaped without altering the appearance of the corpuscle.

(8) *The fate of immature parasites.* Our observations have led us to regard the pyriform type as the mature form of the parasite within the blood. These pyriform parasites after the rupture of the containing corpuscles generally attempt to enter other corpuscles in the manner already described (p. 235). If they do not succeed, after a variable period of activity (3—60 minutes or more), they become motionless in the plasma, and in most cases gradually disintegrate. Sometimes these parasites, after slowly fading, suddenly seem to rupture, as it has been occasionally noticed that in the last stages pale, almost invisible, granular material is protruded from them. Some, however, especially the irregular forms described on p. 243, maintain their normal appearance.

When a corpuscle containing one or more (immature) amoeboid or round parasites ruptures the latter almost immediately become round or quiescent, gradually grow indistinct, and finally disintegrate.

After very careful and prolonged observations on this point we are convinced that all parasites thrown into the plasma by the rupture of the containing corpuscle, which have not reached the pyriform stage, sooner or later disintegrate and die. This is even true of parasites which have almost reached maturity, such as those represented in Diagr. 24. 36. In such cases it might be expected that the few remaining changes would be passed through in the plasma, but this is not the case. Even four fully mature parasites attached together by fine threads do not seem to be able to divide, though the process is readily completed within the corpuscle (Diagr. 27. 18—42).

Our further studies tend to confirm the opinion which we expressed in our last paper (1906, p. 601), that the rounded and irregular free parasites found in organ smears are degenerating forms. Many of the larger types (those illustrated, 1906, Diagr. 10) represent parasites in the early stages of division, which have been liberated by the rupture of the corpuscle containing them, and which, as evidenced by the staining properties of their nuclei, are undergoing degenerative changes.

The extent of the destruction or degeneration of the parasites which takes place in the organs may be seen by reference to a previous paper (Graham-Smith, This *Journal*, v, p. 250. 1905), in which the relative proportions of free and intra-corpuscular parasites in the various organs are given.

In the lungs the degeneration is greatest. Smears show a proportion of 1·5 free parasites to each infected corpuscle. In other organs the degeneration is less. Brain smears show one free parasite to each infected corpuscle, smears of lymphatic glands 1—2·2, kidney smears 1—2·2, liver smears 1—2·5, supra-renal smears 1—4·8, marrow smears 1—8, pancreas smears 1—9, and spleen smears 1—9·5.

A small proportion of the free parasites were no doubt fixed at the time they were passing from a ruptured corpuscle to a normal one. In spite of this fact, however, the figures probably represent fairly accurately the proportion of parasites undergoing degeneration in these organs.

In the peripheral blood the number of free parasites is never so great. "Two and more days before death one free parasite to every 38 infected corpuscles were found. The day before death the proportion was one free parasite to 23 infected corpuscles, and on the day of death one free parasite to 18 infected corpuscles" (p. 253).

Diagram 33 illustrates a prolonged observation on the fate of three intra-corpuscular parasites.

When first noticed 4 minutes after the preparation of the specimen two mature pyriform parasites and one large amoeboid form were noticed within a single red blood corpuscle. During the next $9\frac{1}{2}$ minutes the pyriform parasites remained motionless, but the amoeboid form threw out pear-shaped processes and appeared to be going to divide into four pyriform parasites. Half a minute later, however, the corpuscle suddenly faded and the contained parasites and two small rounded bodies (see p. 245) were expelled into the plasma (14). Within 11 minutes, during which it exhibited a few movements, the amoeboid parasite became rounded (25), and it remained in this condition until the end of the observation, nearly 2 hours after its escape. On the other hand, both the pyriform parasites (A and B) swam actively away and entered normal corpuscles, A within 3 minutes and B within 2 minutes. Both soon assumed a rounded shape, and afterwards showed slight amoeboid movements. Finally they both again became rounded and motionless. Under natural conditions we have no doubt these parasites would have multiplied in the usual manner.

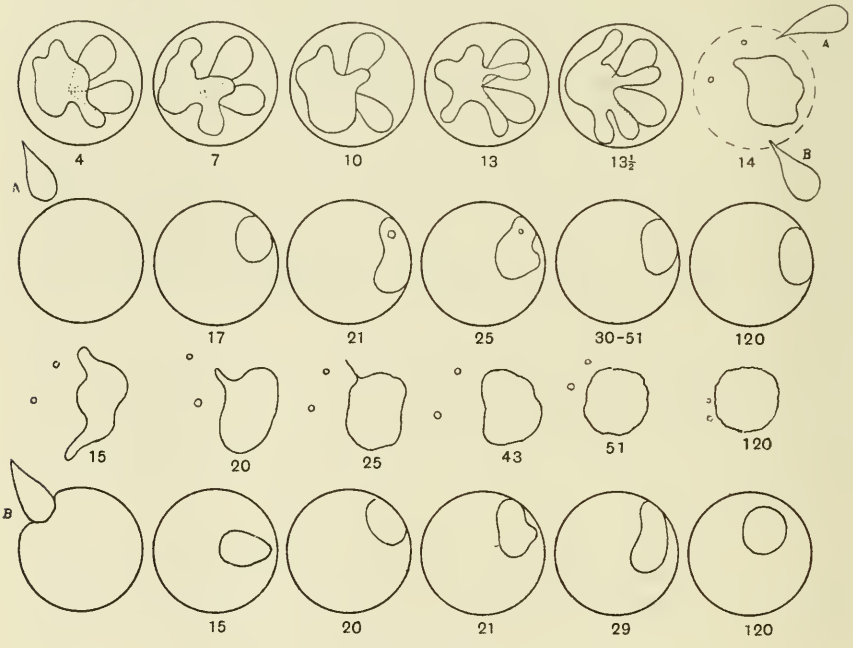


Diagram 33.

(9) *Vacuoles.* In stained preparations clear, well defined, pale areas resembling vacuoles can be seen in a certain number of all

forms of the parasite, but are less common in the actively amoeboid type with pseudopodia. In living preparations vacuoles are also very commonly seen and appear as more or less well defined darker areas of various shapes and sizes within the pale parasites. Plate I, Fig. 1, 11—37, illustrate the changes in the shape of an amoeboid parasite and in the appearance and size of its vacuole during a period of observation lasting 37 minutes.

In the first stage represented, which was drawn 11 minutes after the preparation of the specimen, the vacuole appeared as a well defined large oval area, slightly darker than the parasite. Within half a minute it had contracted to a much smaller size, and its edges had become still better defined. Under these conditions it was a much more noticeable object than before. Shortly afterwards it had moved from the centre to the edge of the parasite, and produced the appearance of an oval notch cut out of the side of the parasite. Subsequently it again moved to the centre of the parasite, and became large and small, and well and ill defined alternately. These changes can be more readily followed by reference to the figures than described.

Vacuoles are indicated in several other figures (Diagrs. 27 and 31) representing living forms, whilst in some they have been intentionally omitted for the sake of clearness.

It is particularly interesting to notice that very large vacuoles are sometimes present in small rounded forms, and that vacuoles of varying size are often present in the symmetrical pear-shaped processes which ultimately develop into mature pyriform parasites. In the latter case the vacuoles still persist in the mature pyriform parasites and are often seen in stained preparations dividing the dense from the loose masses of chromatin.

We have on several occasions made very careful observations on suitable living specimens with Zeiss 2 mm. objectives and high eye-pieces with the purpose of ascertaining whether any evidence of nuclear changes can be obtained. Occasionally darker areas were seen within the protoplasm of some of the parasites, but the significance of such appearances is very doubtful, since with the most careful focussing and adjustment of the light no structure can be made out in many recently liberated pyriform parasites, which, we know from our studies on stained preparations, invariably contain at least one large dense mass of chromatin. Up to the present we have been unable to ascertain anything in regard to the nuclear changes by the study of the living organisms, and the minuteness of the parasites appears at present to be an insurmountable obstacle in the way of solving the problem by this means.

Summary of observations on living blood.

Piroplasma canis has a free and an intra-corpuscular stage in the blood of the dog, and it is during the latter stage that multiplication occurs. This asexual multiplication takes place in one of the following ways:

(1) A free pyriform parasite which has just left a blood corpuscle enters a normal corpuscle and assumes a round form, remaining quiescent for a time. The round body then grows and, after passing through an actively amoeboid stage, again becomes rounded. Two symmetrical processes are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes assumes a pyriform shape and ultimately gives rise to a mature pyriform parasite, which remains for a time joined to its fellow by a thin strand of protoplasm. On the rupture of the containing corpuscle these pyriform parasites become free and enter other corpuscles.

(2) Occasionally by the protrusion of four pyriform processes four mature pyriform parasites are formed from a single amoeboid form.

(3) Sometimes a young rounded intra-corpuscular parasite divides by simple division and gives rise to two amoeboid parasites, which grow and divide by the protrusion of symmetrical processes each into two pyriform parasites, thus giving rise to four mature pyriform bodies within the corpuscle. Sometimes the two amoeboid parasites undergo the processes of division simultaneously, but not infrequently one is considerably in advance of the other.

(4) It is possible that occasionally a red blood corpuscle is invaded either simultaneously or at different times by two pyriform parasites, each of which undergoes the changes described above.

(5) Several pyriform bodies within a single corpuscle are produced by the division in the manner described of one or more amoeboid parasites.

All parasites which have not reached the mature pyriform stage, when the containing corpuscle ruptures, rapidly degenerate and die in the plasma. The same is true of mature pyriform parasites, which do not, after becoming free, soon enter other corpuscles.

In observations made on living preparations many of the intra-corpuscular parasites, after a longer or shorter period of activity, come to rest as rounded forms near the edges of the corpuscles. This appears to be due to the unfavourable conditions and probably does not occur in the living body.

In an earlier paper (Graham-Smith, 1905, p. 265), a table is given showing the relative frequency of the occurrence of infected red blood corpuscles containing various numbers of parasites, compiled from a number of observations made on organ smears and blood films. Excluding corpuscles containing only single parasites 27,088 infected corpuscles were counted, and of these 26,305 (96·38 %) contained even numbers of parasites (22,286 with two parasites, 3397 with four parasites), and 783 (2·89 %) odd numbers. The mode of multiplication accounts for this disparity.

(C) The nuclear changes accompanying division.

Aided by the knowledge we had gained of the significance of the various forms assumed during division by the living parasites, we made very careful and extended studies on stained preparations.

In preparing these specimens thin smears were made on thoroughly clean glass slides. Some were fixed (1) by drying in the air, (2) others were immediately plunged into boiling absolute alcohol and a few were fixed either by (3) immersion in a mixture of 1 c.c. of strong formalin to 10 c.c. of alcohol, or (4) by being placed first in formalin vapour for 5 seconds and then immersed in alcohol.

By the first two methods extremely good preparations were obtained, but the other two did not yield as satisfactory results.

All preparations were stained by Giemsa's stain, but the dilution and the period of staining was varied according to the result which was desired.

In some cases dehaemaglobinised films were used, as the finer chromatin strands are more easily studied when the pink tint imparted to the corpuscles by the presence of the haemoglobin is removed.

In our last paper (1906, p. 590) we pointed out that in many parasites several distinct masses of chromatin can be recognised, a large, dense, compact mass, and a lightly staining, irregular, loosely packed or reticulated mass or masses, which had not been previously described. These masses have been regularly noticed in our later observations, and we have been able to determine their relations to each other.

By most observers the dense mass has been described as the nucleus, but owing to the peculiar manner in which the chromatin division takes place, we prefer at present not to give it any definite name.

Schaudinn (1904, p. 438) briefly called attention to the presence of a

third small compact punctiform chromatin body, which he called the blepharoplast. Lühe (1906, p. 47) confirmed this observation, and in our last paper (1906) we gave several figures illustrating it.

In our later observations, however, we have seldom encountered this body, and do not consider that it is a structure of any significance in the division of the parasites. In many cases it seems to represent the loose chromatin in a condensed form.

At first our studies were confined to the final stages of the development of two pyriform parasites from the period when the two symmetrical processes are first protruded to the completion of two mature parasites as shown on pp. 237—238 in Diagrs. 24 (30—83) and 25 (21—45). All these stages can be readily identified in stained preparations, and the nuclear changes, peculiar to each, easily studied. We are, therefore, in a position to state without hesitation that the division of the chromatin takes place in the manner described below.

When the actively amoeboid stage is past and the parasite assumes a round form just before the protrusion of the symmetrical processes (Diagr. 25. 21), there projects from the large dense central mass of chromatin a thin strand of chromatin which bifurcates near its extremity. Each branch ends in a small knob of dense chromatin (Diagr. 34. 7, Plate II, Figs. 6, 33). In specimens which have developed further and show the earliest indications of the symmetrical processes it is found that a terminal branch of the chromatin thread passes into each (Diagr. 34. 8, Plate II, Figs. 7, 18, 34). In specimens at a later stage, showing larger processes, the same condition is noticed (see p. 258, Diagr. 34. 9, Plate II, Figs. 7, 8, 18, 19). At this stage a strand of chromatin passes out of the main mass in the body of the parasite and bifurcates at the base of the processes. From the point of bifurcation a strand passes down into each process and ends, usually near its extremity, in a small knob.

By the time the parasite has reached the trilobed stage (Diagr. 24. 33) the main mass of chromatin has altered its position, and (drawn down as it were to the bifurcation by the contraction of the connecting strand) is now situated at the base of the processes. The strand, which connected the main nuclear mass to the branches passing into each process, consequently disappears at this stage (Diagr. 34. 10, Plate II, Figs. 9, 10, 11, 18).

By the time the parasite has reached the stage of division corresponding to that shown in Diagr. 24. 36, the main mass of chromatin has almost divided and is represented by two incompletely separated

masses lying side by side, from each of which a strand passes into a pear-shaped process (Diagr. 34. 11, Plate II, Figs. 12, 35).

A little later the two incompletely separated masses just mentioned move apart, but remain connected by a thin strand (Diagr. 34. 12, Plate II, Figs. 13, 36).

By the time the parasite has reached the condition shown in Diagr. 24. 40, the connecting strand between the two large masses of chromatin has disappeared and each of these has definitely moved into the neck of the pyriform process (Diagr. 34. 13, Plate II, Fig. 20). During these last stages (Diagr. 34. 12, 13) another change has been taking place. The originally thin strand of chromatin, which extended down each process, gradually loses its sharp outline and usually becomes broken up, especially at its knob-like extremity, and now has a loose appearance. At this stage, therefore, the chromatin in the now almost completely formed pyriform parasite has a comet-like appearance, the head of the comet near the apex of the parasite being represented by the dense mass of chromatin and the tail by the loose mass (1906, Plate XI, Fig. 31; Plate II, Figs. 13, 15).

During the further stages in the formation of the two pyriform parasites, and even after their liberation from the corpuscle, as free parasites, the chromatin remains in the same condition (Diagr. 34. 13, 14, 15, Plate II, Fig. 46).

Sometimes that portion of the chromatin strand which projects from the main mass retains its thread-like appearance and only its distal extremity becomes loose in structure (Diagr. 35. 13, 14, above; 1906, Plate XI, Fig. 23; Plate II, Figs. 39, 40). At other times this portion almost completely disappears and there is scarcely any connection to be seen between the dense and loose masses (1906, Plate XI, Figs. 25, 28; Plate II, Figs. 40, 50). More rarely that portion of the chromatin which is usually loose and net-like in structure is represented by a number of small dense granules (Plate II, Fig. 47). Up to the present we have no reason to consider that the variations in the final disposition of the chromatin have any special significance.

When a vacuole or vacuoles are present in the rounded parasite the resulting pyriform parasites are usually also vacuolated. Under these conditions the processes which give rise to these pyriform parasites contain one or more vacuoles at all stages with the possible exception of the very earliest. It is interesting to note that the chromatin almost always has a very definite relation to the vacuoles. In the stages represented in Diagr. 34, Figs. 7 and 8, and Plate II, Figs. 6 and 7, the chromatin

strand runs close to the margin of the vacuoles. During the development of the processes the chromatin strand in each is also closely connected with the vacuoles. When one vacuole is present the strand usually runs round at least one-third and often nearly three-quarters of its circumference (Diagr. 34. 10, Plate II, Figs. 9, 10, 20, 35), and not infrequently, when the strand ends some distance beyond the vacuole, it has a flange-like process passing off at an angle and helping to surround the vacuole (Plate II, Figs. 9, 11, 20). When two vacuoles are present the chromatin strand usually runs between them, often sending off the flange-like processes just mentioned to partially surround one or both vacuoles (Plate II, Figs. 12, 13, 36).

When a vacuole persists in the mature pyriform parasite it either lies at one side of the loose chromatin mass (Diagr. 34. 14, Plate II, Fig. 40) with the tail of the latter curving round it, or separates the dense from the loose mass (1906, Plate XI, Fig. 29; and Diagr. 1. Fig. 4), or most rarely is almost completely surrounded by the chromatin (Plate II, Fig. 37).

Consequently, in regard to the disposition of the secondary masses of chromatin, at least six well-marked varieties of mature pyriform parasites are found, (1) those showing a large loose mass of chromatin connected to the main rounded dense mass near the pointed extremity, (Plate II, Fig. 15; 1906, Plate XI, Figs. 30, 31, 18, and Diagr. 1. Fig. 3; Diagr. 3. Fig. 2; Diagr. 6. Figs. 8, 9, 10); (2) those showing the loose mass near the blunt extremity connected to the main mass by a thin strand of well defined chromatin (Plate II, Fig. 39; 1906, Plate XI, Fig. 23, and Diagr. 3. Fig. 12 and Diagr. 6. Fig. 10); (3) those in which the dense and loose masses are separated from each other (Plate II, Fig. 40; 1906, Plate XI, Fig. 20, 25; Diagr. 1. Figs. 1, 2, 5, 14; Diagr. 3. Figs. 3, 4, 6, 7); (4) those in which that portion of the chromatin which is usually loose in structure is represented by dense granules (Plate II, Fig. 47; 1906, Plate XI, Fig. 24; Diagr. 3. Figs. 8, 10, 22); (5) those in which a distinct vacuole is more or less interposed between the dense and loose chromatin (1906, Plate XI, Fig. 29, and Diagr. 1. Fig. 4); and (6) those in which the loose chromatin is more dense in structure than usual, and is closely applied to the dense mass (rare forms) (Plate II, Fig. 48; 1906, Diagr. 3. Fig. 1).

The secondary masses of chromatin are usually arranged in a similar manner in the two mature pyriform parasites derived from a single amoeboid parasite, but this is not always the case.

Although the various arrangements of the secondary masses of

chromatin in the mature pyriform parasites probably do not represent differences in function, and may perhaps be accounted for by the movements of the protoplasm during their formation, they are of importance when considering the distribution of the chromatin in the small rounded forms to which the pyriforms give rise after entering fresh corpuscles.

The nuclear changes we have hitherto described can be followed without difficulty owing to the characteristic appearances of the parasites during these phases of division, but those changes which occur between the entry of the parasite into a normal corpuscle and the end of the actively amoeboid stage cannot be followed so easily. This is owing to the difficulty of deciding what stage a small rounded intra-corpuscular parasite has reached when seen in a stained preparation.

We confidently believe, however, that in the following pages these changes are correctly described.

Shortly after its entry into a normal corpuscle the pyriform parasite assumes a rounded shape. At this stage the chromatin is arranged in exactly the same manner as it was in the free pyriform parasite (Diagr. 34. 2). Within a short time, while the parasite is still small, the loose chromatin moves towards the main mass (Plate II, Fig. 1), and finally the whole becomes aggregated into one large dense mass (Plate II, Fig. 2). The parasite in some cases has become actively amoeboid before the process is completed. The varying periods at which complete fusion occurs is well seen by reference to a diagram in our last paper (1906, Diagr. 9) illustrating several amoeboid parasites, some with single masses of chromatin (Figs. 3, 7, 11, 12, 13, 15, 18), and others with both loose and dense masses (Figs. 6, 14, 17). During this process different appearances will be seen according to the differences in the arrangement in the pyriform parasites which entered the corpuscles.

Diagr. 34. Figs. 2, 3, 4, 5 (Plate II, Fig. 23; 1906, Diagr. 2. Fig. 4) schematically represent these changes when the entering parasite possesses a vacuole. Diagr. 35. Figs. 1—5 (upper row, Plate II, Figs. 22, 25, 26) represent the changes when the main mass of chromatin of the entering parasite is attached to the loose mass by a thin strand of chromatin, and the lower row of figures the changes when the loose and dense masses are connected without the interposition of a strand.

Subsequently, in most cases towards the end of the actively

amoeboid stage, the large mass of dense chromatin, formed by the fusion of all the chromatin substance, divides into two, often unequal, masses, which remain joined by a thin strand (Diagr. 34. 6; Plate II, Figs. 3, 4, 5; and 1906, Diagr. 2. Figs. 2 and 16). Later the smaller of these two masses again divides. By this means the peculiar Y-shaped chromatin figure already described is formed, which is found in a parasite about to protrude the two symmetrical processes which ultimately give rise to the new pyriform parasites (Diagr. 34. 7, Plate II, Fig. 6).

From this point to the completion of division the nuclear changes have already been described (p. 252).

When the young parasite divides by simple division, and gives rise to two small amoeboid parasites, the process is preceded by the complete division of the chromatin mass (Diagr. 36. 4; Plate II, Figs. 16, 17; 1906, Diagr. 2. Fig. 13).

Subsequently each separate parasite behaves exactly like a single dividing form (Diagr. 36. 6—10; Plate II, Figs. 18, 19, 20).

The arrangement of the chromatin in the irregular forms of intracorpuscular pyriform parasites deserves a brief description.

The very large pyriform type shown in Plate II, Fig. 42, usually possesses very little chromatin which is situated in small widely separated masses (see also 1905, Plate 9, Fig. 13).

The form showing a spike-like process (Plate II, Fig. 43) generally exhibits similar masses of chromatin to those seen in mature pyriform parasites.

The large irregular parasites represented in Plate II, Fig. 44, each possess a single mass of dense chromatin and an extensive network of loose chromatin. These forms are of particular interest, because in one instance similar shaped organisms were seen to give rise to several pyriform parasites (p. 240).

When suitable opportunities occur for the observation of pyriform parasites lying on their edges, it can frequently be seen that the dense mass of chromatin causes a distinct prominence on the surface of the organism. Such a condition is illustrated in Plate II, Fig. 46.

It has generally been asserted that polychromatophile degeneration is not found in canine piroplasmosis, and the condition is undoubtedly very rare. In one of our cases, however, a large number of corpuscles were affected, one of which is represented in Plate II, Fig. 48.

(D) The complete cycle of development within the blood.

In a previous paper (1906), and on page 235 of the present paper, we have fully described, with the aid of drawings made during the observations, the multiplication processes which we have studied in the living blood of dogs suffering from acute canine piroplasmosis. Since, in the course of very prolonged and careful investigations, we have never seen indications of any other methods of reproduction than those we have described, we believe that, whatever the number of parasites produced within single red blood corpuscles, the process in all cases is essentially similar. Further, we believe that multiplication never occurs outside the corpuscles, and that all immature parasites, which are liberated by the rupture of the corpuscles which contains them, and all mature pyriform parasites, which fail to enter fresh corpuscles degenerate and die.

The nuclear changes which occur in the parasites during the process of growth and division within the red blood corpuscles, and the various arrangements of the secondary masses of chromatin in the mature forms, have been described in the last section.

The results of all our observations on living and stained specimens are summarised in the present section by the aid of schematic figures which indicate the principal changes in form which the parasites undergo in the processes of multiplication and passage from one corpuscle to another and the nuclear changes which accompany them.

Description of Diagrams 34—36.

Diagram 34. Fig. 1 (p. 258), shows a free pyriform parasite about to enter a normal red blood corpuscle which it is indenting (1906, Plate XI, Figs. 17, 18, etc.). The parasite contains a vacuole, and possesses a single dense mass of chromatin connected with a loose mass near its blunt end by a thin strand.

In Fig. 2 the parasite is represented as having entered the corpuscle and become rounded in shape, while the chromatin still retains its original disposition (Plate II, Fig. 23).

In Fig. 3 the parasite has grown and its vacuole has enlarged, but the chromatin has not undergone any alteration.

In Fig. 4 the parasite has still further enlarged, and the loose mass of chromatin has been drawn up to, and become condensed close to the dense mass (Plate II, Fig. 1).

In Fig. 5 the whole of the chromatin has fused, and a secondary loose mass can no longer be differentiated (Plate II, Fig. 2).

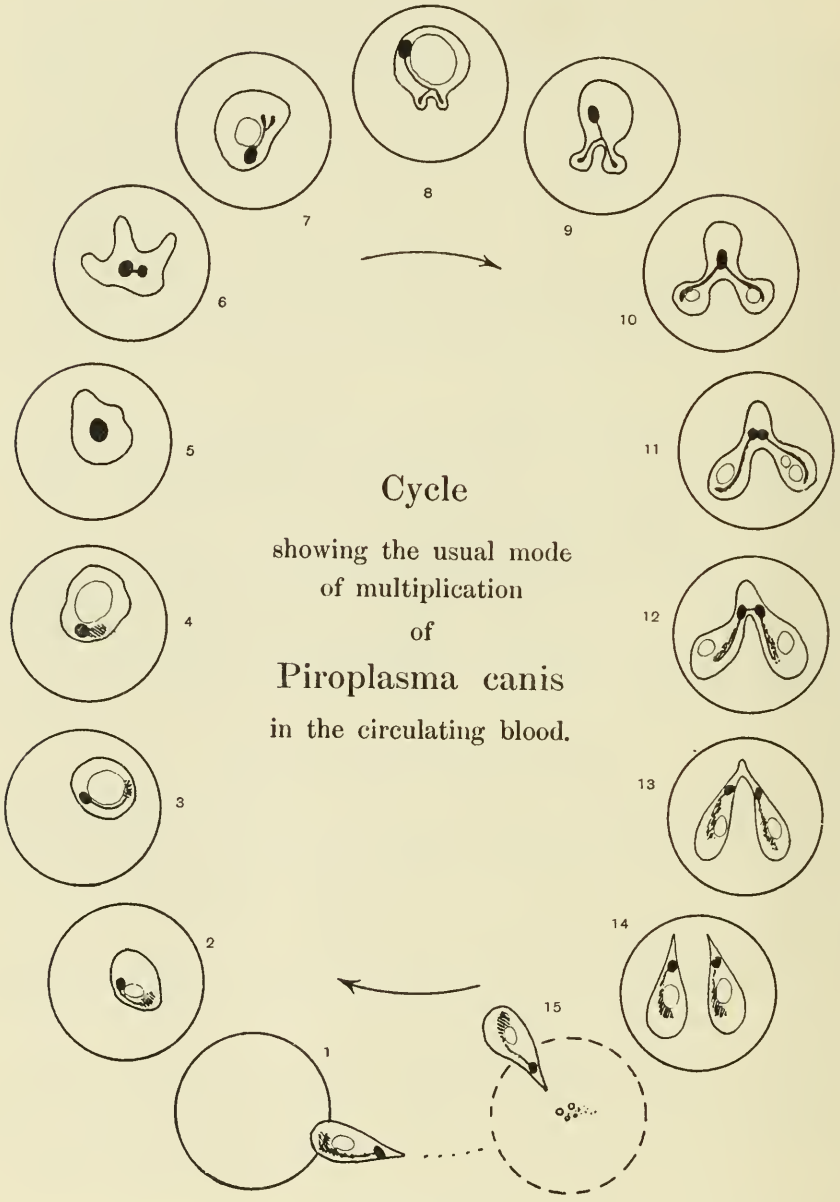


Diagram 34.

In Fig. 6 the parasite is represented in the amoeboid stage with three pseudopodia. The single chromatin mass has by this time divided into two unequal sized masses, connected together by a thin strand (Plate II, Figs. 3, 4, 5; 1906, Plate XI, Fig. 8). For the sake of simplicity the long amoeboid stage is represented by one figure only, and for the same reason the vacuole has been omitted in this and the previous figure. During the earlier stages represented in Figs. 1—4 and the later stages shown in Figs. 7—15, the vacuole, when present, is closely related to the chromatin, which almost invariably lies along its margin. In those stages, however, in which the whole of the chromatin is gathered together into a central mass, no special relation to the vacuole has been noticed.

In Fig. 7 the parasite is represented in the rounded quiescent stage after the cessation of the active amoeboid movements. At this stage the two masses of chromatin shown in the last figure have moved apart and in the smaller has again divided. The three main masses thus produced are still connected together by a thin strand, which runs from the main mass close along the edge of the vacuole and eventually bifurcates to send a branch to each of the divisions of the smaller mass (Plate II, Figs. 6, 33). At this stage the parasite shows no processes.

In Fig. 8 two small symmetrical processes have been protruded by the parasite, each supplied with one of the divisions of the smaller chromatin mass (Plate II, Figs. 7, 18, 19, and 34).

In Fig. 9 the processes have enlarged, but the general arrangement of the chromatin remains the same as before (Plate II, Fig. 8).

In Fig. 10 the trefoil stage (see Diagr. 24. 33) is represented. By this time the main chromatin mass has altered its relation to the body of the parasite and to the rest of the chromatin. It is no longer situated at a distance from the processes, but has moved to a position near their bases. During this movement the chromatin strand, which connected the main mass to the two branches passing into each process, shortens and disappears, so that finally the latter branches project directly from the main mass (Plate II, Figs. 9, 10, 11, 18, 20).

An attempt has also been made to indicate the relation of the chromatin to the vacuoles which appear in the processes about this stage. This relationship has already been described (p. 253), and is better seen by reference to Plate II, Figs. 9, 11, 12, 13, 20, 35, 36.

In Fig. 11 the processes have still further enlarged at the expense

of the body of the parasite, and the main mass of chromatin situated near their bases shows signs of division (Plate II, Figs. 12, 35).

In Fig. 12 the division just mentioned is represented as nearly completed, but the resulting masses are still connected by a thin strand. At this stage a change takes place in the appearance of the strands of chromatin passing down the processes. They lose their definite contour and become transformed into masses of loose chromatin with a reticular structure (Plate II, Figs. 13, 36).

In Fig. 13 the original body of the parasite has almost completely disappeared, and the whole of the chromatin has passed into the processes. The dense masses resulting from the division mentioned in Figs. 11 and 12 become completely separated, and finally take up their positions near the tapered extremities of the processes (Plate II, Fig. 20).

In Fig. 14 the completely formed pyriform parasites, resulting from the division of the single parasite which entered the corpuscle (Fig. 1), are shown. Each possesses a dense mass of chromatin near its pointed extremity, from which a tail of loose chromatin passes towards the blunt extremity. The latter is closely apposed to the margin of the vacuole (Plate II, Figs. 15, 19, 20, 38, 39, 40).

In Fig. 15 the escape of these two parasites is indicated, and one is represented as passing into another corpuscle (Fig. 1). The discontinuous line represents the contour of the ruptured corpuscle. In the centre of the latter some granular matter is shown, which may either represent residual protoplasm cast off by the parasite during division, or the remains of the stroma of the corpuscle (see p. 246).

Diagram 35 supplements Diagr. 34, giving two of the variations in the disposition of the secondary or loose chromatin, which may occur subsequent to the stage represented in the latter in Fig. 12.

Fig. 12 (right-hand central Fig.) is in all respects similar to Fig. 12, Diagr. 34, except for the fact that no vacuole is represented. The upper row of figures represents the appearances which are seen when the terminal portions only of the strands of chromatin, which pass down the processes, are changed into reticular masses. In Fig. 14 the completely formed parasites of this type within the corpuscle are represented (Plate II, Figs. 39, 40), and in Fig. 15 the rupture of the corpuscle and the escape of the parasites, one of which is in the hour-glass condition while passing through the corpuscular envelope

(see p. 245). Figs. 1, 2, 3, 4, and 5 show the gradual condensation of the chromatin into a single mass after the entry of one of the parasites into a fresh corpuscle (Plate II, Fig. 23).

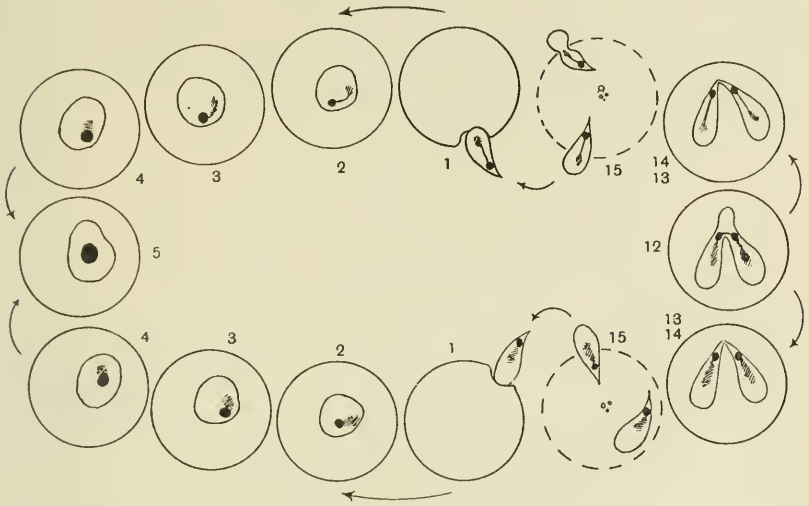


Diagram 35.

In the lower row of figures the same series of events are represented in parasites without vacuoles, in which the secondary mass of chromatin forms a loose mass closely related to the dense mass (Plate II, Figs. 15, 45).

The subsequent changes are similar to those shown in Diagr. 34. Figs. 6—15.

Diagram 36 indicates two methods by which four pyriform parasites may be produced within a single corpuscle.

In Fig. 1 a single pyriform parasite is shown entering a corpuscle.

In Fig. 2 the same parasite is represented after its entry with the corpuscle. In Fig. 3 the chromatin has become condensed into one mass. Up to this point the parasite has behaved in the same manner as that represented in Diagr. 34. Figs. 1—5. In Fig. 4 the parasite is shown in the act of dividing into two small rounded parasites (Plate II, Fig. 16), the nucleus having already divided. In Fig. 5 the resulting two small round parasites, each with a single dense mass of chromatin, are shown (Plate II, Fig. 17). In a previous paper (1905, Plate IX, Figs. 3, 4, 5, 6, and 7) the appearances during

these stages have been well represented. Consequently, we have not reproduced similar figures in the plate accompanying this paper. After this each parasite behaves in the same manner as the one shown in Diagr. 34. Fig. 6 corresponds to Diagr. 34. Fig. 5; Fig. 7 to Diagr. 34. Fig. 9; Fig. 8 to Diagr. 34. Fig. 10; and Figs. 9 and 10 to Diagr. 34. Figs. 12, 13, and 14 (Plate II, Figs. 18, 19, 20)[1]. The escape of the four pyriform parasites is shown in Fig. 11 (Plate III).

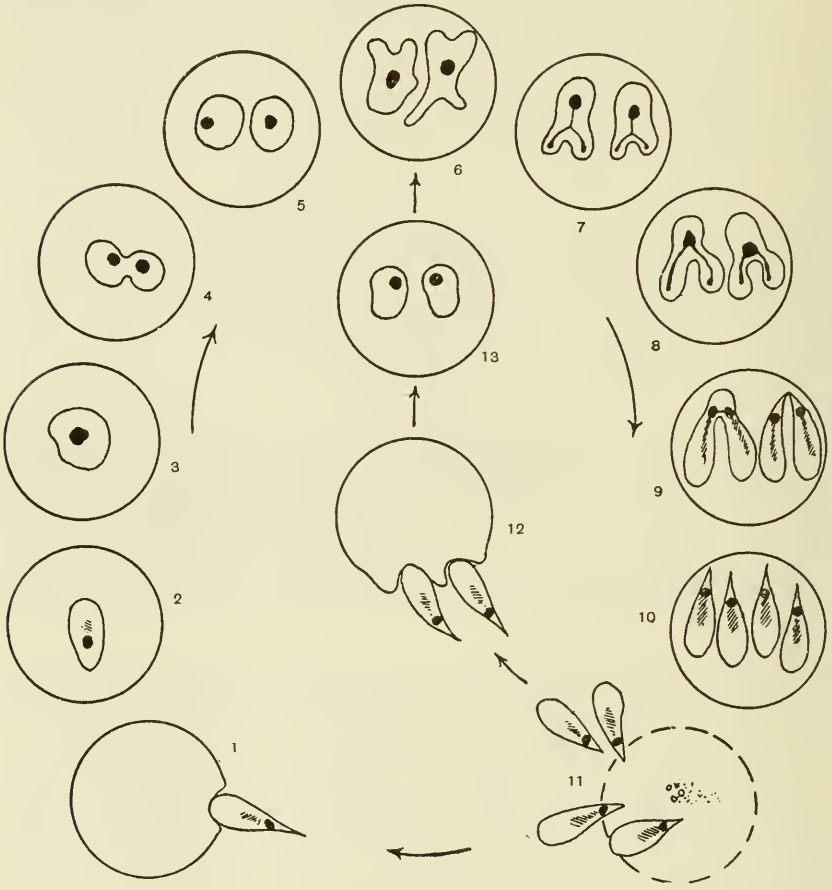


Diagram 36.

[1] Various stages in this process are clearly represented in some of the figures of our last paper (1906). In Diagr. 5. Fig. 6, two small parasites are shown, one with a single mass of chromatin, and one with two masses joined by a strand. In Fig. 6 two parasites are shown each with two masses joined by a strand. In Figs. 9 and 10 later stages are represented.

We believe that this is the usual mode of formation of four parasites within a single corpuscle.

Although we have never observed the entry of two parasites into a single corpuscle, it does not follow that such an event never occurs within the dog's body, when the corpuscles are closely packed together in the slow current of capillaries. In such a case each parasite would probably soon become rounded and retract its chromatin into a single mass (Fig. 13), subsequently these parasites would, we assume, behave in the same manner as two small parasites derived by division from a single parasite (Figs. 6—11).

Conclusions.

With the exception of a few observations on points which have little or no bearing on the development of the parasites, we have brought to a conclusion our experiments on that part of the life cycle of *Piroplasma canis* which is passed within the blood of the dog. Our observations on the living blood alone, conducted at all stages of the disease and at all hours of the day between 9 a.m. and midnight, have occupied more than 550 hours of careful study.

We therefore think that we are justified in considering that none of the common phases at any rate, which can be followed under experimental conditions, have escaped our notice. As we have already pointed out, our observations lend no support to any of the theories of development which have hitherto been put forward (see 1906, pp. 641—643). Most of those appearances which give rise to erroneous impressions have been briefly recounted in a previous paper (1906, pp. 604—610) and certain others are given in the appendix to this paper (p. 268).

Our observations on the mode of development of single free parasites, which enter red blood corpuscles, into two mature parasites, and on the methods by which several pyriform parasites are produced in single red blood corpuscles are summarised at length in this paper (p. 250).

We, however, briefly recapitulate the former process here:

A free pyriform parasite enters a normal red blood corpuscle and rapidly assumes a rounded form. It then enlarges and passes through an actively amoeboid stage, at the end of which it again becomes rounded. After a short period of quiescence in this condition it protrudes two symmetrical processes, which rapidly grow and become

pear-shaped. The protoplasm of the parasite flows into these processes, and its body consequently gradually diminishes until it is represented by a minute rounded mass to which the pyriform processes are attached. Eventually this also disappears, and finally two mature pyriform parasites are left, which are joined together for a time by a thin strand of protoplasm. After a variable time these parasites are liberated by the rupture of the corpuscle, and swim away to enter fresh corpuscles and repeat the process.

Occasionally a single rounded intra-corpuscular parasite by the protrusion of several processes, such as have just been described, gives rise to four or more mature parasites, or a single parasite divides into two small rounded parasites, each of which produces two pyriform parasites.

Under experimental conditions all parasites, which are liberated by the rupture of the corpuscles containing them before they have reached the mature pyriform stage and all mature pyriform parasites which fail to quickly enter fresh corpuscles, disintegrate and die.

Observations on stained preparations lead us to conclude that from this cause a great destruction of the parasites takes place in the living body, especially in the organs. Under the conditions of observation many parasites fail to become fully developed in the corpuscles, and come to rest as rounded forms, but this probably does not occur as frequently in the living body.

Aided by the knowledge we had gained by the study of living preparations, we have made very prolonged and careful examinations of stained specimens, and have been able to work out the nuclear changes which accompany development. These changes have been summarised at length in section (**D**) (p. 257).

Briefly these changes are as follows:

The free pyriform parasites possess a mass of dense chromatin near their pointed extremity and a secondary mass of loose chromatin extending towards the blunt end, which may be arranged in various ways. When the parasite becomes rounded within a corpuscle the original arrangement of chromatin is retained for a time. Gradually the two masses become approximated, and either before or during the amoeboid stage become fused into a single dense mass. This mass later divides, but the resulting masses remain united by a thin strand of chromatin. Just before the protrusion of the symmetrical processes, one of the latter masses again divides in such a manner that a peculiar Y-shaped chromatin figure is formed. This consists

of a large dense mass from which a thin strand projects, which bifurcates at some distance from the large mass, sending strands to two small masses. When the processes are formed one of these smaller masses passes into each, still remaining united with the larger mass. At a late stage in the division of the parasites the main mass of chromatin also divides, and a portion passes into each process, ultimately giving rise to the dense mass in the mature pyriform parasite. The smaller mass and the connecting strands give rise to the secondary mass.

When a small round intra-corpuscular parasite divides into two small parasites, the process is preceded by the simple division of the chromatin. In the subsequent development of these parasites the chromatin behaves in the manner described above.

We have never observed any forms which could be regarded as gametes (see 1906, p. 640, footnote).

DESCRIPTION OF PLATES I—III.

PLATE I. Has been described sufficiently in the text under section (A), pp. 233—234.

PLATE II. The specimens illustrated in this plate were painted with the greatest possible care to depict as accurately as possible every structure which was visible.

When a suitable specimen had been found under the microscope (2 mm. Zeiss aprochromatic oil immersion and 6, 8, 12, and 18 compensating oculars with artificial light) it was sketched as accurately as possible by one observer (G.-S.). The specimen was then painted by the other observer (N.) and finally the two figures were compared and the details discussed. Unless both observers were in complete agreement upon the details of every structure the specimen was rejected.

We can, therefore, confidently assert that the figures accurately represent the appearances seen in preparations carefully stained by Giemsa's method.

Figs. 1—15 show the stages in the formation of a pair of pyriform parasites from a single rounded form.

Fig. 1 shows a small intra-corpuscular form in which the loose chromatin has approached the dense mass.

Fig. 2 shows a later stage in which all the chromatin has fused into a single dense mass.

Figs. 3, 4, and 5 show three stages in the division of the single mass into two masses joined by a thin strand, which soon become widely separated.

Fig. 6 shows the parasite after the completion of the active amoeboid stage. One mass of chromatin has again divided giving rise to two small knob-like masses joined by strands to the single strand which projects from the main mass.

Fig. 7 represents an early stage in the formation of the symmetrical processes. One of the knob-like masses seen in the last figure is situated in each process, but still remains connected in the same manner as before to the main mass. While the body of the parasite possesses a large vacuole a small one is contained in each process.

Fig. 8 represents a condition in which the processes have enlarged, but the general disposition of the processes remains the same as before.

Fig. 9 represents a still more advanced condition. Here the main mass of chromatin has taken up its position near the bases of the processes and is directly connected to the strands passing into each.

Figs. 10 and 11 represent further stages in the growth of the processes. The relations of the chromatin remain unaltered.

Fig. 12 represents the earliest stage in the division of the main mass of chromatin. It is now represented by two rounded closely connected masses.

Fig. 13 represents the stage in which the body of the parasite has almost disappeared and the divisions of the main mass of chromatin have moved apart but are still connected by a thin strand. The secondary mass of chromatin is no longer thread-like but is loose and widely distributed.

Fig. 14 represents a slightly more advanced condition in which the chromatin in one process is no longer connected to that in the other.

Fig. 15 represents two mature intra-corpuscular parasites each with a rounded dense mass of chromatin near its apex, and a loose mass extending from it.

Figs. 16—20 represent the stages in the formation of two pairs of pyriform parasites.

Fig. 16 represents a small parasite in the act of dividing.

Fig. 17 shows the completion of such a division resulting in two small rounded parasites within one corpuscle (see also 1905, Plate IX, Figs. 3, 4, 5, 6, and 7, and description, p. 248).

Fig. 18 represents an early stage in the production of four pyriform parasites from two amoeboid parasites. The left-hand specimen is slightly further advanced than the right-hand one.

Fig. 19 represents another example of the same process. The corpuscle contains two mature parasites which have developed from one amoeboid parasite, as well as an amoeboid parasite in an earlier stage of development.

Fig. 20 represents a corpuscle containing two almost mature parasites, a parasite in an advanced stage of development into two parasites, and a rounded form which has not yet completed the condensation of its chromatin.

Fig. 21 represents a rounded intra-corpuscular parasite with a large dense mass of chromatin and a smaller separate mass ("blepharoplast" of Schaudinn and Lühe).

Fig. 22 represents a young intra-corpuscular parasite before the condensation of the chromatin. This was evidently derived from the form of parasite shown in Fig. 39, since it possesses a dense mass of chromatin attached to a loose reticular mass by a thin strand.

Fig. 23 represents a parasite in the same stage as that shown in the preceding figure. Here a vacuole, surrounded by thin strands of chromatin, is interposed between the dense and the loose chromatin. This form is derived from the kind of parasite shown in one of our previous papers (1906, Plate XI, Fig. 29, or Diagr. 1. Fig. 4).

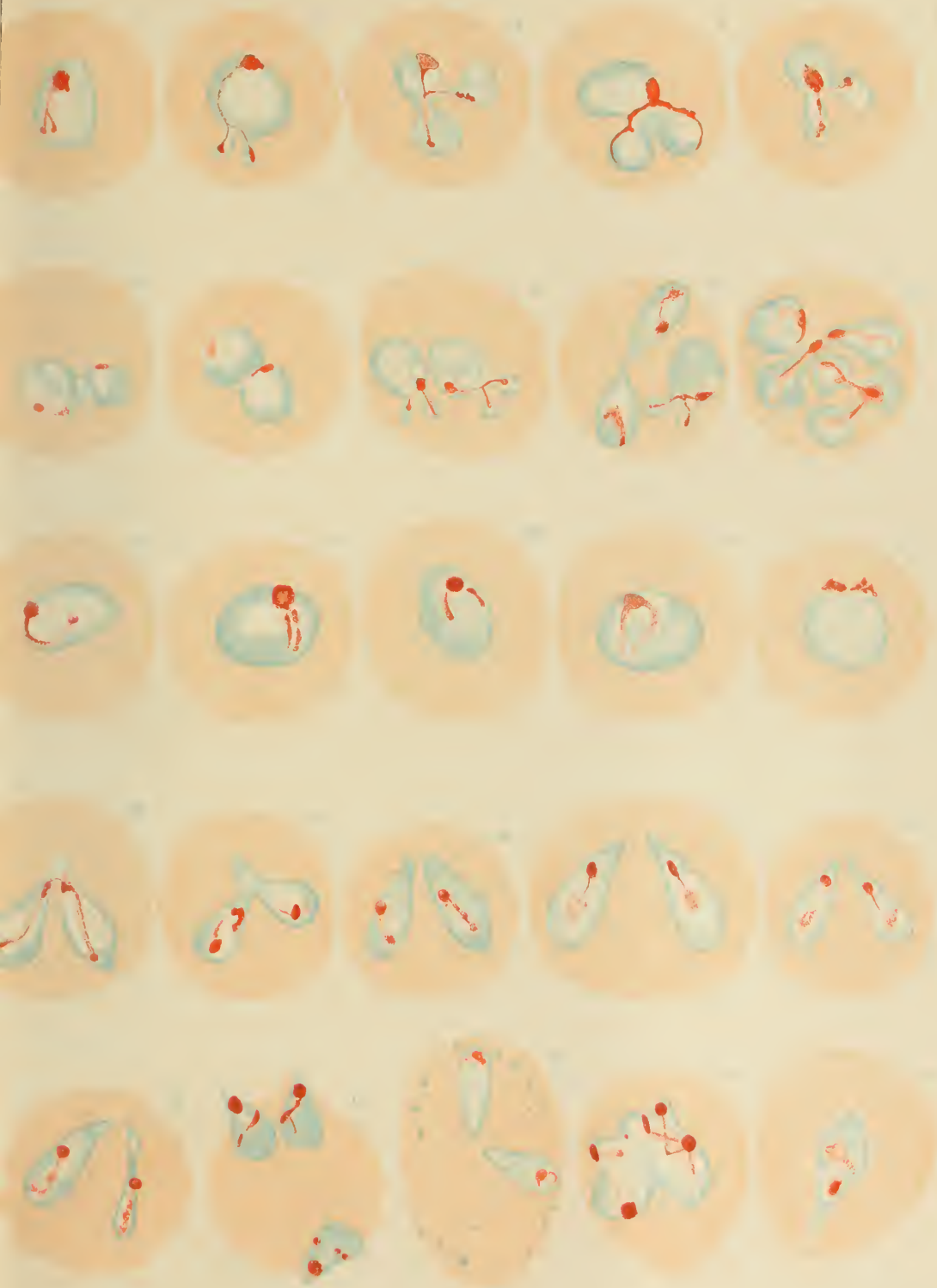
Figs. 24, 25, and 26 probably represent varieties of the type shown in Fig. 22.

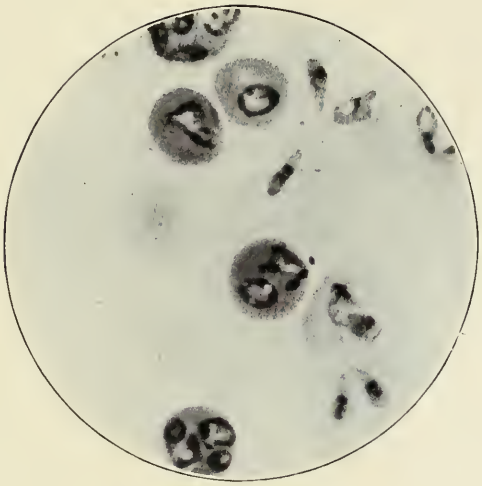
Figs. 27 and 28 probably represent stages in the condensation of the chromatin in intra-corpuscular parasites derived from such pyriform parasites as are seen in Figs. 37, 38, 47; 1906, Plate XI, Fig. 24.

Fig. 29 appears to represent a later stage in the development of the form shown in Fig. 23, when the vacuole has enlarged and caused the chromatin surrounding it to become thinned out.

Fig. 30 probably represents an abnormal type of the division seen in Fig. 3.

Fig. 31 probably represents an early stage in an atypical form of the division of the





second mass of chromatin derived from the division shown in Fig. 3 and corresponds to the stage represented in Fig. 6. The division results in one small mass moving away from the other, which remains in connection with the original strand.

Fig. 32 represents a more advanced stage in the same atypical mode of division.

Fig. 33 shows an unusual form of the condition represented in Fig. 6.

Fig. 34 represents a more advanced stage of the process seen in Figs. 31 and 32. One of the processes which has formed, contains a strand of chromatin with a small mass at its extremity, while the mass which should have moved into the other process retains its original position. We think that on further growth the second mass would pass into its process, and develop the usual connecting strand. If this took place a parasite resembling that shown in Fig. 7 would be formed.

Fig. 35 represents a parasite in the same stage as that shown in Fig. 12. It shows extremely well the encircling of the vacuoles in the processes by the chromatin.

Fig. 36 represents a parasite in the same stage as that in Fig. 13 but with a slightly different arrangement of chromatin.

Fig. 37 represents a parasite in the same stage as that shown in Fig. 14. The chromatin is disposed in a very unusual manner.

Figs. 38, 39, and 40 represent mature intra-corpuscular parasites with various arrangements of their secondary masses of chromatin.

Fig. 41 represents a parasite in the same stage as that shown in Fig. 11. The secondary mass of chromatin is in an unusually concentrated condition.

Figs. 42, 43, and 44 show irregular forms of mature intra-corpuscular parasites. Those seen in Fig. 42 are typical in shape, but of very large size and possess very little chromatin.

One parasite in Fig. 43 has a spike-like process projecting from its side. The dense and loose masses of chromatin are very close together. The irregular pyriform parasites shown in Fig. 44 are particularly interesting, because in one instance similar shaped organisms were seen to give rise to several regular pyriform parasites. Each possesses a single mass of dense chromatin and an extensive network of loose chromatin.

Fig. 45 represents a common condition. The corpuscle contains two fully mature parasites and one rounded form in an early stage of development.

Fig. 46 shows two pyriform parasites in a slightly crenated corpuscle. One of the parasites is seen on edge, and shows extremely well the projection caused by the dense mass of chromatin.

Fig. 47 shows three intra-corpuscular parasites in the act of escaping. Two of these have pushed the envelope of the corpuscle before them.

Fig. 48 represents two parasites in a corpuscle which shows polychromatophile degeneration.

Fig. 49 represents a parasite with a complicated arrangement of the chromatin, probably about to give rise to several pyriform parasites.

Fig. 50 represents a free pyriform parasite lying *on* a normal corpuscle. The organism is surrounded by a white halo (see Fig. *a*, Plate I) and has produced a further distortion of the surface of the corpuscle which is evidenced by two white lines running from the pointed end of the parasite to the margin of the corpuscle.

PLATE III. Photograph of a stained preparation of the blood of a dog taken shortly before death. All the corpuscles are infected. In one instance (see line on the right) the parasites have been fixed in the act of escaping. Two free parasites have already left the corpuscle and two are still within the remains. On the side on which the parasites have escaped the corpuscle has lost its haemoglobin, but on the other side some is still left, and the contour of the corpuscle can be distinctly seen. Another corpuscle (in the

field) has almost completely vanished and the two pyriform parasites which have escaped appear widely separated. We are greatly indebted to Mr Max Poser, of the Firm of Carl Zeiss, London, for this remarkable photograph which he has kindly taken from one of our specimens.

Appendix.

In certain of our earlier observations we were surprised to occasionally observe dumb-bell-shaped bodies, which showed well marked movements, as well as rounded bodies with long flagella-like processes often ending in a collection of minute knobs. Despite the most careful observation we did not at that time succeed in tracing the origin of these bodies, nor could we account for the fact that they were sometimes present in one film made from a drop of blood and not in another film made from the same drop.

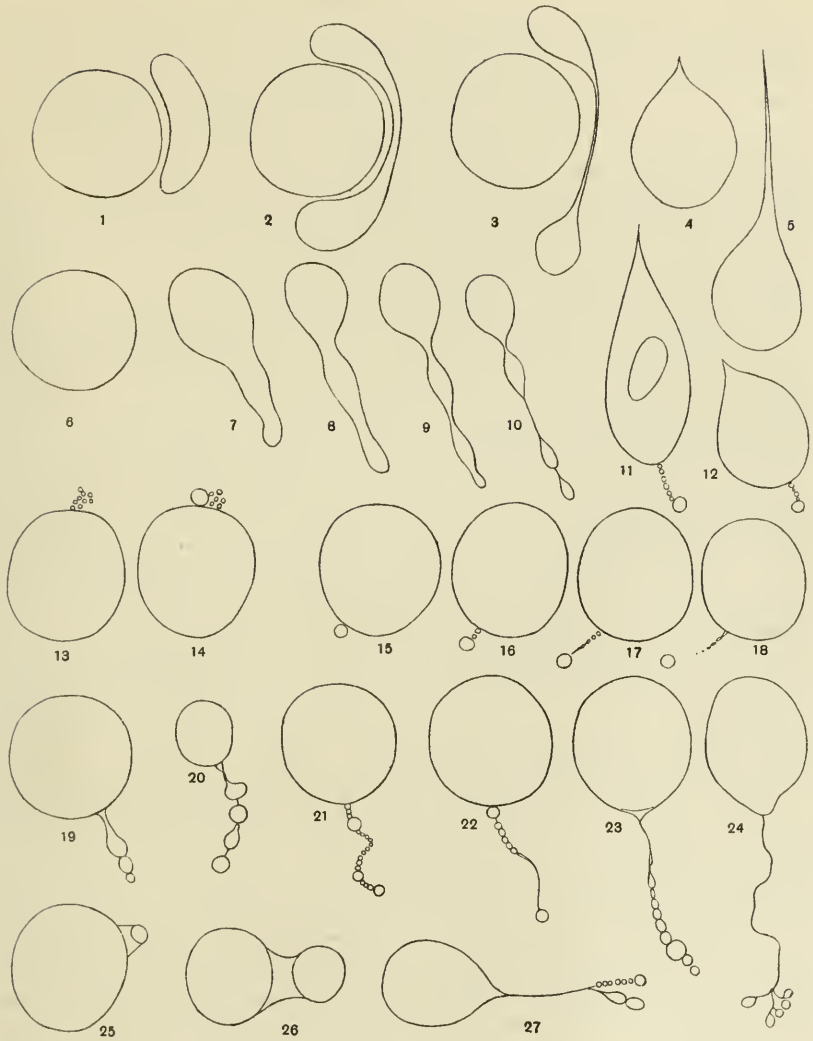
In our last paper (1906, p. 627; Diagr. 21. Figs. 3, 4, and 5) we figured and described some of these bodies which we saw in specimens prepared during the last few hours of life, and which we regarded as free forms of the parasites. At the same time we were careful to point out that we had not been able to follow their development.

Since that time we have made further studies on this question and have succeeded in demonstrating that these bodies are not parasites, but degeneration products of the red blood corpuscles.

Similar appearances had apparently been noted by Durham[1] in his work on trypanosomes, and, although in the books we have consulted, no references are made to the subject, the changes about to be described are doubtless known to physiologists. Consequently, we do not claim that these observations describe hitherto unknown phenomena. We merely take this opportunity of again calling attention to the changes, of which we were completely ignorant, which occur in blood corpuscles under certain conditions. The appearances to which these changes give rise caused us much trouble, and are probably unknown to many workers on these subjects.

When a fresh specimen of normal human blood is mounted by placing a drop on a clean glass slide, and covering it with a cover-glass and ringing the latter round with vaseline, the corpuscles appear as round, pale discs. If the temperature of the preparation is gradually raised in a thermostat to 50° C., the leucocytes become rounded and motionless, but the red corpuscles do not alter. When the temperature

[1] Mr H. E. Durham informs us that he remembers having seen such bodies, but we have been unable to find any reference to them in any publication.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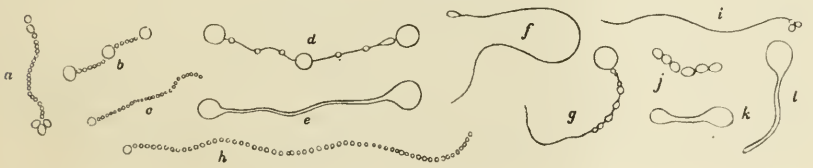
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Diagram 37.

is raised to between 51·5° C. and 52·5° C., however, very marked changes generally occur. Some of the corpuscles suddenly elongate, or show one or more minute round bubble-like protrusions (Diagr. 37. Figs. 4, 5, 6—10, 13, 14, 15). Not infrequently, one of these protrusions is suddenly projected for a considerable distance, but still remains attached to the corpuscle by a thin strand (Figs. 16, 17, 18, 24). Figs. 15, 16, 17, 18 illustrate a case in which a small round mass was first protruded (15), and then rather rapidly moved from the side of the corpuscle while retaining its attachment by means of a strand. At other times the corpuscle elongates and the point seems to stick to the glass and the corpuscle moves away producing the same result (Figs. 6—10). Fig. 6 shows a normal corpuscle which suddenly became elongated (7). The pointed extremity seemed to stick to the glass while the main mass moved away, eventually giving rise to a chain of four unequal sized masses attached to each other by a thin strand (10). Occasionally several small round or elongated masses form the extremity of the projection, and may be united to each other by thin filaments (Figs. 24, 27). These processes are continually in motion, moving from side to side.

Much more commonly the strand is not uniform, but shows swellings of various sizes and shapes along its course. These swellings may be elongated or rounded, large or small, and regularly or irregularly distributed. These various types may alternate in the same strand. Figs. 11, 12, 19, for example, show short irregular protrusions, Fig. 20 a longer example of the same type, Fig. 21 a long strand with many minute and three larger swellings, and Figs. 22 and 23 more irregular types. Sometimes a corpuscle moves from its place and impinges against another which is apparently fixed to the glass. In this case the moving corpuscle may become flattened, and eventually elongated, so as to curve round the other. By this time most of the corpuscular substance is accumulated at the ends, and the central portion is reduced to a thread. In this way a dumb-bell-shaped body is formed (Figs. 1, 2, 3).

Figs. 1—3, 6—10, 15—18 illustrate examples in which the various stages were drawn during the process of formation.

In every case the protrusion of substance causes a more or less considerable diminution in the size of the corpuscle, depending on the quantity of substance extruded (Figs. 6—10). Not infrequently the corpuscles shrink to half their original size, or even less.

Sometimes the corpuscle divides into two rounded masses of equal

or unequal size. These may remain united by very thin, almost invisible, substance (Figs. 25, 26), or become completely separated. They may either remain in this condition, or one or both of them may extrude material in the manner described.

Further, it very frequently happens that the long extended filaments break off either near the original corpuscle or at some distant point, giving rise to free filaments, which show active "wriggling" movements due to the molecular vibrations of the constituent particles.

A few of these are represented in Figs. *a*—*l*. Some of these bodies, owing to their colourless appearance and active movements closely simulate parasites.

If the specimen is suddenly raised to 52·5° C. the changes just described are extremely well marked. Very few normal corpuscles can be found, and the blood corpuscles are converted into innumerable small round bodies, such as are shown in Figs. 13, 14, mixed with moving filaments, belonging to all the types shown in Figs. *a*—*l*.

A lesser degree of heat combined with mechanical injury, such as that caused by pressure, brings about similar changes.

Other changes of less interest from our point of view take place at slightly higher temperatures, for example, some of the corpuscles become irregular in shape and then suddenly fade, others split into fragments, and others become crenated.

In the few observations we have made on the blood of different species of animals we have noticed that they differ in regard to the temperature at which this change takes place.

The blood corpuscles of guinea-pigs and rabbits withstand a greater degree of heat than human blood, and do not break up in this manner below 54° C. Normal dog's blood acts in the same manner. We believe that the blood of dogs suffering from piroplasmosis reacts at a slightly lower temperature. Nucleated blood corpuscles (fowl, frog, Fig. 11) behave in the same way.

The bodies which we have briefly described cannot be easily mistaken, when their origin, movements, and general configuration have once been carefully studied.

In transferring a drop of blood to a slide we made use of a thin platinum loop recently sterilised by heat. In some instances the loop, although apparently cool, must have retained sufficient heat to act on those corpuscles with which it came in contact. Consequently, in the preparations subsequently made, most of the corpuscles appeared normal, but here and there a small degenerated form with a long

filament attached, or a completely detached motile filament (Figs. *a*—*l*) was seen. Owing to their motility and lack of colour these bodies were at first mistaken for flagellated forms of the parasite. The fact that such forms could not be found in stained preparations, and sometimes only occurred in one out of two films made from the same drop of blood, soon made us realise that they had probably nothing to do with the parasites, but were artificially produced. Nevertheless, we had some difficulty in determining their true nature.

In all our later experiments described in the present paper, this source of error has been carefully excluded.

We hope that this brief note may be of assistance to those who are working on similar subjects.